in S-Phase (BrdU incorporation and PCNA immunohistochemistry) or by the number of mitotic cells (WCMC). Questions/issues addressed by the consultant in regard to these negative data include:

1) Appropriateness of the biopsy site

2) Appropriateness of endpoints. Is there colonic epithelial cell hyperproliferation? Is cell proliferation a valid technique?

3) Is there a risk of colon cancer?

1) **Appropriateness of the Biopsy Site**

In study W-144999 the sponsor elected to do rectal biopsy to describe colonic events. The consultant is being asked to assess if this biopsy site is appropriate. Once again the non-absorbed TG are neutral and relatively innocous and therefore intracolonic fat should not be expected to induce proliferation at a selective colonic site that would be missed if one biopsies the rectum only. So, if any effect is expected (on proliferation) this would be due to orlistat, or orlistat-serine or orlistat small peptide. And the proliferation effect would depend on where the compound is absorbed [what segment of the colon and if there are differences in the absorption, depending on colonic segment]. But there is no information on the site (if any) of colonic absorption. On the contrary, there are data suggesting that, all in all, the compound (in original or derivative form) is poorly absorbed from the gut. In addition, the rectum represents the easiest, most convenient site and it is also a place where colonic neoplasia takes place.[31] Multiple studies have shown that ca. a third of patients with hyperplastic polyps in the rectum will have adenomatous polyps proximally [E. Achkar and M.V. Sivak, Gastroenterology 101:1145 (1991)]. In patients with rectosigmoid adenomas, p53 protein accumulation in rectosigmoid adenomas appears to be a predictive marker for proximal advanced neoplasms [G.V. Papatheodoridis et al., Gastroenterology 110:A574 (1996)]. Based on this reasoning, the consultant reaches the conclusion that the biopsy site seems appropriate. However, there are a number of other data and considerations that need to be kept in mind when deciding whether the rectal mucosa is representative. These are briefly mentioned below. Specific examples are provided when appropriate.

The colon is important for the absorption of water and electrolytes, a process which occurs predominantly in the cecum. Although the rectum is not a usual site for absorption of ingested nutrients, drugs introduced by this route (such as salicylates or steroids) may be absorbed there. The colon constitutes three segments that are functionally distinct. In the proximal part of the colon, the major motility patterns are retrograde annular contractions, producing antiperistalsis, which retains and mixes the content in the cecum and ascending colon for long periods of time. In the middle part, the motility patterns are annular contractions, which divide the fecal mass and move them very slowly toward the rectum. In the most distal segment, there are strong contractions, which move caudad (excreted by stimulation of

pelvic nerves). These motility patterns would tend to promote drug absorption in the cecum and ascending colon. In this region of the colon the contents also exhibit the lowest viscosity compared with the rest of the colon and rectum. The rectum is a unique segment capable of distending, holding gas under pressure and behaving as a reservoir. Regional differences in large bowel SCFA metabolism, absorption, and utilization have been identified but their implications related to pathological conditions have not been established. Studies on isolated human colonocytes have shown a greater degree of n-butyrate utilization in distal than in proximal colon, despite similar rates of absorption. Morphological regional differences also have been identified [A.P. Rabassa and A.I. Rogers, Amer. J. Gastroenterol. 87:419-423 (1992)].

2) Appropriateness of Endpoints. Is There Epithelial Cell Hyperproliferation? Is Cell Proliferation a Valid Technique?

For corectal cancer, primary prevention is directed at inhibiting the onset of colorectal cancer. Secondary prevention is directed at inhibiting colorectal carcinogenesis through endoscopic surveillance of high-risk populations and removal of preneoplastic lesions. But before primary prevention can take place, it is essential to identify endogenous and exogenous factors involved in the process of colorectal carcinogenesis. Given the likely involvement of dietary factors in colorectal cancer (as outlined in many publications), it is reasonable to study the luminal contents of the colon as well as options to reduce its carcinogenic potential based on effects on colonocytes.

In study W-144999, three techniques were used to measure proliferation in biopsy specimens from colorectal mucosa by quantifying proliferation with a numerical parameter, the labeling index (LI). In the first technique, tissue labeling was done by incorporating a marker (bromodeoxyuridine = BrdU; 3 biopsy samples) into cells. BrdU is a halogenated pyrimidine [thymidine]-analogine incorporated into DNA, which can be quantified by fluorescent of chromophoric quenching of dyes bound to DNA or with antibodies to BrdU. These technologies have been used since the 1970s as tools for measuring DNA synthesis in isolated chromosomes and in cells and tissues. In the second technique, specific proteins (i.e. proliferating cell nuclear antigen = PCNA; 3 biopsy samples) associated with proliferating cells were assayed. PCNA is a nuclear antigen expressed in the late GI and S phase of proliferating cells, which is neither species nor tissue species [W.J. Adams et al., Eur. J. Surg. Oncol. 19:332-335 (1993)]. In the third technique, the number of arrested metaphases was counted (i.e., whole crypt mitotic index = WCMI; later changed so that whole crypt mitotic count = WCMC was reported; 2 biopsy samples). This WCMC technique measures the number of mitoses in microdissected crypts. The important contribution of the WCMC is that major obstacles of traditional methods (BrdU, PCNA, [^{3}H]thymidine) are overcome by crypt isolation [H. Konishi et al., Gastroenterology 108:A491 (1995)].

In study W-144999, for measurements and analysis of PD parameters of mucosal cell turnover, crypts were compartmentalized so that 5 crypt compartments were used for the BrdU and the PCNA labeling indices, respectively and 10 crypt

compartments for the WCMC value. The data were analyzed descriptively by crypt and total (mean, range and standard deviation) for measurements from biopsy samples collected at baseline (day -7) and treatment (day 43), as well as for change (Δ) from baseline. Student's t-tests were used to test for a significant difference ($p<0.05$) between the orlistat and PL treatment groups for each crypt compartment and for the total (sum of all crypt compartments) for BrdU, PCNA and WCMC. Confidence intervals around the ratio of follow-up total value were calculated for each of the 3 biomarkers using Fieller's Theorem [M: Risio, J. Cell. Biochem., Suppl.16G:79-87 (1992)].

The endpoints used in study W-144999 were appropriate and the cell proliferation evaluations were well executed. Total crypt score for BrdU and PCNA, before (sponsor's Fig. 1) and after treatment (sponsor's Fig. 2) were expressed as labeling index-percent, for the total crypt scores. The total crypt score for WCMC was expressed as the number of cells in mitosis for the total crypt. There were no statistically significant differences between the orlistat and PL treatment groups before or after treatment. There were no significant changes (treatment-baseline) induced by orlistat when compared to PL for the WCMC in the ten crypt compartments (sponsor's Fig. 3). Actually, this Fig. showed a tendency for the mean change in compartments 2 and 3 (close to the base=compartment 1) to be lower with orlistat but this difference was not significant. As shown in sponsor's Fig. 4, there was no significant differences in the changes (treatment-baseline) induced by orlistat when compared to PL for the BrdU incorporation in the five crypt compartments. Actually, there was a tendency for the mean change to be lower towards the middle of the crypt (compartments 2 and 3) following orlistat treatment, but this difference was not significant. The changes (treatment-baseline) induced by orlistat in PCNA immunochemistry in the five crypt compartments (sponsor's Fig. 5) were very similar (the two lines are almost superimposable) to those changes induced by placebo.

Is there colonic epithelial cell hyperproliferation?

Based on the use of appropriate endpoints and the detailed description of the results above, it can be concluded that, under the experimental conditions used in study W-144999, there was no cell hyperproliferation. Furthermore, in their Table 8, the sponsor presented correlation coefficients, probability, number of observations for change in selected parameters measured in fecal material and fecal water against change in biomarkers (BrdU, PCNA, WCMC). There were no significant correlations for any of these comparisons.

Is cell proliferation a valid technique?

The study of epithelial cell proliferation represents an evaluation of an intermediate biomarker of colorectal cancer. Although its meaning is not universally accepted, colorectal epithelial cell proliferation accompanies the first stage of colorectal carcinogenesis and epithelial cell proliferation is increased in high risk subjects for colorectal cancer. Studies that evaluate modulation of colorectal epithelial and luminal factors associated with cancer development have included, as in study W-144999, cytolytic activity of fecal

water, bile acids, epithelial cell proliferation and SCFA. Of these, SCFA were not evaluated in this PD study. But the results from the other three PD parameters do not seem to give reasons for concern.

Recently, the validity of cell proliferation as a major risk factor for colon and other cancers has been questioned [E. Farber, Cancer Res. 55:3759-3762 (1995)]. It is axiomatic that cell proliferation plays an important and even critical role in many steps in cancer development. It can also be stated that cell proliferation with altered control is the prime property that is the first characteristic phenotype feature of a malignant neoplastic cell population. Indeed, much information accumulated during the last 15 years reveals that cell proliferation is often associated with carcinogenesis in rodents and humans.[32] When added to the colonic lavage, sennosides, which chronic use has been associated with increased colon cancer risk, induce acute massive cell loss by apoptosis, causing a decrease in crypt length, followed by increased cell proliferation and inhibition of apoptosis to restore cellularity [B.A.P. van Gorkom et al., Gastroenterology 110:A608 (1996)].[33] But the overall accuracy of proliferative parameters in discriminating between "normal" and "high risk" for colon cancer is rather low; in a recent study, abnormal proliferative patterns were found in only half of patients with adenomatous polyps, and the most predictive feature, an upward extension of the proliferative zone, was also present in 23% of normal controls [G. Marra et al., Gastroenterology 106:A412 (1994)]. Furthermore, as pointed out by Ward et al. [Envir. Health Persp. 101(Suppl. 5):125-136 (1993)], an increasing number of examples can be found that suggest cell proliferation is often not associated with carcinogenesis. The group of Earnest et al. have reported a lack of correlation between colonic epithelial cell proliferation and drug-induced inhibition of colon cancer in rats treated with azoxymethane. Treatment with CA significantly increased colon tumors, cancer and the WCMC but not BrdU labeling index. Both piroxicam and UDCA decreased tumor and cancer development. However, the labeling in piroxicam and UDCA-treated rats did not correlate with neoplasm tumor development. These results do not support that labeling index in normal appearing rectal mucosa is a surrogate endpoint biomarker for predicting effects of cancer chemoprevention drugs. They also suggest that suppression of cancer development by piroxicam and UDCA does not occur through a pathway primarily affecting epithelial cell proliferation [Gastroenterology 108:A463 (1995)]. Also, sulindac (an NSAID) exhibits tumor incidence and causes tumor regression but does not act on proliferation in dimethylhydrazine treated rat colon [W. Fischbag and J. Rheinländer, Gastroenterology 108:A468 (1995)]. In summary then, chronic cell proliferation does occur [in colon cancer] but, in itself, it does not seem to be associated with an increased risk for carcinogenesis. In conclusion, clinically, cell proliferation seems to be out of vogue.

3) Is There a Risk of Colon Cancer?

No one can, of course, answer this question with certainty. This is because the available information to assess carcinogenesis in humans is very incomplete. But the 1997 most accepted view is that cancer of the colon is a **multistage carcinogenicity process**. The cause of colorectal cancer is now

widely accepted to be the accumulation of mutations in specific genes controlling cell division, apoptosis, and DNA repair [K.W. Kinzler and B. Vogelstein, Cell 87:159-170 (1996)]. In the case of orlistat's long-term administration it appears that the colon cancer concern might be at least partially counteracted by several pieces of information, including:

1. Orlistat does not seem to have mutagenicity or genotoxicity potential. This statement is based on negative results of a battery of short-term assays.[1] [This information is important because of the observation that most mutagens are also carcinogenic.]

2. There are also nonmutagenic carcinogens for which there are no predictive assays and for which conventional extrapolations to potential effects in human being may not apply. The most obvious biological activity for many of these nongenotoxic agents is the induction of cell proliferation. But, according to the results in study W-144999, orlistat does not seem to induce cell proliferation in obese subjects given a hypocaloric diet and the compound at the recommended doses for six weeks.

3. Because the unhydrolyzed TG being offered to the colon is structurally normal, this situation is like in other malabsorption syndrome, especially pancreatic insufficiency. No effects of the fat on colonic architecture are expected.

4. Although higher than before treatment, the amount/concentration of FFAs being offered to the colon does not increase much with orlistat. Not much cytotoxic effect due to this low amount/concentration of these FFAs is expected.

> [On the other hand, the increase in unhydrolyzed TG and some FFAs would be accompanied by an increase in fecal water. It is axiomatic that steatorrhea will eventually induce diarrhea.]

5. Orlistat's PD effects result in a significant decrease in total BAs, particularly DCA not only in the solid but - most importantly - the liquid phase of the stool. Orlistat shares this PD effect with compounds now being tested in the prevention of colorectal cancer, such as UDCA.

6. Orlistat inhibits the secretory phospholipase A_2, an enzyme involved in the production of arachidonic acid, which is a substrate for the production of PGs and LTs [A.J. Watson and R.N. DuBois, Lancet 349:444-445 (1997)].[2]

[1]
- Ames tests (± metabolic activation) in tester strains TA97, TA98, TA100, TA102, TA1535, TA1537 and TA1538.
- Mammalian cell (V79/HPRT) gene mutation assay (± metabolic activation)
- Unscheduled DNA synthesis in primary cultures of rat hepatocytes (UDS assay)
- Clastogenesis *in vitro* in human peripheral lymphocytes (± metabolic activation)
- Chromosome aberration assay *in vivo*, in mice (mouse micronucleus test)

[2] How this enzyme modifies tumor susceptibility is unknown, but it is interesting that it is secreted in Paneth cells into the intestinal lumen, where it could digest dietary fats or where its bactericidal effects could alter bacterial flora [S.S. Harwig et al., J. Clin. Invest. 95:603-610 (1995)].

Recent findings indicate that COX-2 may play an important part in the development or maintenance of adenomas and that increased COX-2 expression could result directly from an inability of adenomatous polyposis coli to carry out its normal function. In man, use of NSAIDs has been linked to a 40 to 50% reduction in relative risk for colorectal cancer [R.N. DuBois et al., Gastroenterol. Clin. North Amer. 25:773-791 (1996)].[24]

7. Finally, due to a decrease in the intestinal absorption of liposoluble vitamins with orlistat treatment, higher than normal amounts of vitamin A, D, E and K are being offered to the colon. Possible associations between vitamins and cancer incidence have been discussed in numerous reviews. None of the vitamin-cancer relationships are proved and only a few associations can be said to be supported by a reasonably sound body of evidence. But lower consumption of vitamin A (dietary retinol and carotenoids plus supplements) appears associated with increased risk of colonic adenomas [J.W. Kikendall, Gastroenterology 106:A401 (1994)]. Vitamin D and retinoid x receptor gene expression in human colonic mucosa and tumors provides a rational basis for therapy with 1, 25 $(OH)_2$ vitamin D_3 analogs. The latter have been shown to inhibit azoxymethane-induced colonic tumorigenesis [R.K. Wali, Gastroenterology 108:A550 (1995)] and to prevent adenomas from progressing to carcinomas, probably through protein kinase C isoforms [R.K. Wali et al., Gastroenterology 111:118-126 (1996)]. To exert its salutory proposed cytoprotective effects, the liposoluble vitamins (or, more likely, their derivatives) would need to be in the liquid phase of the stool, in contact with the colonic epithelial cell. This hypothesis is worth testing. Again, more liposoluble vitamins offered to the colon does not seem to represent a situation of concern.

IV. RECOMMENDATIONS FOR REGULATORY ACTION

This consult review assessed the results of a pharmacodynamic study (Protocol W-144999) in obese subjects given a hypocaloric diet (1900 Kcal/day; ca. 30% fat) and orlistat, at 20 mg t.i.d., the proposed recommended dose for six weeks. Analysis of total weight, total fat, free fatty acids total and individual bile acid content and calcium and pH in the fecal material as well as free fatty acids, total and individual bile acid concentration and pH in the fecal water did not reveal findings of concern under orlistat treatment, in comparison to those induced by a placebo control. A detailed analysis of the evidence, from rectal biopsies, based on crypt compartment analysis of three biomarkers (BrdU, PCNA and WCMC) together with numerous pertinent publications allows the following conclusions a) the biopsy site (rectum) is appropriate; b) the endpoints to assess cell proliferation (quantification of proliferation with a numerical parameter, the labeling index and compartmentalization of crypts), are also appropriate; c) under the experimental conditions used in this trial, orlistat did not induce colonic epithelial cell proliferation. Although cell proliferation as a biomarker for colon cancer seems out of vogue, the consultant believes that the lack of changes in cell proliferation and the significant decrease in deoxycholic acid in both the aqueous and solid phase of the stool, give the general feeling that we are not talking about a dangerous situation here. But in reality, the

available PD information is incomplete and the long-term effects of orlistat on colonic architecture are not known. Since obesity is a lifetime disease, treatment with orlistat is expected to last years during which time there is ample opportunity for the occurrence of presently unforeseeable mucosal colonic changes. The following is recommended:

1. Post marketing surveillance for people to whom the compound may be most dangerous in the long term. These include those with risk factors (i.e. low fiber diet), those with predisposing conditions (i.e. ulcerative colitis greater than 10 years) and those with premalignant lesions (i.e. dysplasia, adenomatous polyps, bilious adenomas, familial polyposis, previous colon cancer and schistosomiasis).

2. The orlistat induced decrease in DCA in the liquid and solid phase of the stool may be potentially beneficial. The compound is neither genotoxic nor mutagenic. The sponsor should consider doing animal experiments to answer the following questions, in a progressive fashion:

   a) Does orlistat produce growth inhibition of malignant colonic epithelial cells *in vitro and/or in vivo*?

   b) Does orlistat prevent cancer in an animal (rat) model?

   c) Is orlistat a chemopreventive agent in colorectal cancer in man?

/S/ [redacted] March 6, 1997

Hugo E. Gallo-Torres, M.D., Ph.D.

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HFD-510/EColman
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1. • E.K. Weibel et al., J. Antibiot. (Tokyo) 40:1081-1085 (1987)
   • E. Hochuli et al., Ibid 40:1086-1091 (1987)
   • P. Hadváry et al., JBC 266:2021-2027 (1991)

2. According to a review by Carey and Hernell [Seminars GI Dis. 3:189-208 (1992)] in healthy human adults, the level of enzyme secreted into the intestinal lumen was calculated to be in 1,000-fold excess of what would be required for hydrolysis of the 100 g of TG ingested daily. However, the pancreas is not fully developed at birth, resulting in much lower postprandial luminal levels of pancreatic enzymes during the neonatal period, especially in preterm newborns. Furthermore, the physiological TG-substrate of breast-fed newborns, i.e., the human milk fat globule is a poor substrate for colipase-dependent lipase even in the presence of required cofactors, i.e., colipase and bile salts (vide infra).

3. In contrast to the lipase itself, colipase is secreted as a proform, procolipase or colipase 101 that is cleaved by trypsin to colipase 96 [B. Borgström et al., FEBS Lett. 108:407-410 (1979)].

4. The principal function of colipase is to induce tight-binding of pancreatic lipase to the emulsion interface in the presence of physiological levels of bile-salts. Furthermore, the active colipase is capable of penetrating phospholipid-covered TG emulsions in contrast to procolipase. It has been claimed that the N-terminal pentapeptide cleaved off by trypsin and named enterostatin may be involved in control of satiety [C. Erlanison-Albertsson and A. Larsson, Biochimie 70:1245-1250 (1988)].

5. Results of studies by Khouri et al. [Gastroenterology 96:848-852 (1989)] indicate that in adult patients with pancreatic insufficiency, the fecal TG content does not differ from the controls. However, a fivefold to sixfold increase in fecal FA content in patients with pancreatic insufficiency was revealed. [As patients with maldigestion do not excrete an excess of undigested TG, it is not possible to differentiate maldigestion from malabsorption by quantifying fecal TG and FA].

6. Depending on the animal species and substrates used for characterization, many names have been used to denote what now appears to be the same enzyme, i.e., pancreatic esterase, cholesterol esterase, cholesterol ester hydrolase, carboxylic ester lipase, retinyl ester hydrolase, lysophospholipase, etc. During evolution, this lipolytic enzyme seems to have been a primitive lipase and preceded the colipase dependent pancreatic lipase. As with BSSL, CEH shows lack of specificity toward both fatty ester chemistry and physical-chemical state of substrates and hydrolytic rates decrease in the rank order, micelles are hydrolyzed faster than emulsions, which are hydrolyzed faster than liquid crystals [M. Lindström et al., BBA 959:178-184 (1988); K. Reue et al., J. Lipid Res. 32:267-276 (1991)].

7. This is the only "lipase" secreted in proenzyme form. The active enzyme catalyzes the specific hydrolysis of *sn*-2 FA ester linkages in a variety of phosphoglycerides but it is without effect on sphingolipids, which appear to be hydrolyzed by an enzyme or enzymes on the brush-border of absorptive cells -- possibly the poorly characterized lactase-ceramidase complex. Pancreatic phospholipase A_2 has an absolute requirement for Ca^{2+} ions that bind in a 1:1 stoichiometry to substrate and enzyme at its active site. The enzyme rapidly hydrolyzes phospholipid preferentially in micelles, but also in liquid crystals and on emulsion surfaces.

8. • Y. Huang and D.Y. Dui, J. Lipid Res. 31:2029-2037 (1990)
   • H.J. Aho et al., Int. J. Pancreatol. 5:123-134 (1989)
   • P. Lechêne de la Porte, BBB 920:237-246 (1987)

9. • M.S. Bosner et al., Biochemistry 85:7438-7442 (1988)

10. B. Bergström and H.L. Brockman. Lipases. Amsterdam, Elsevier Science, pp. 1-527 (1984)

11. [Refo #86 in the Carey and Hernell review (locus cited) (1992)].

12. • C. Güzelhan et al., Int. J. Obesity 15(Suppl.1):29 (1991)
   • J. Hauptman et al., AJCN 55:309S-313S (1992)

13. H.B. McMichael, Digestion and malabsorption of fat, in Bouchier IAD, Allen RN, Hodgson HJF (eds): Textbook of Gastroenterology, London, England, Balliére, pp 367-375 (1984).

14. J.S. Trier et al., Gastroenterology 75:307-316 (1978)

15. G. Neale: Bacteriology of the small gut and bacterial overgrowth, in Bouchier IAD, Allen RN, Hodgson HJF (eds): Textbook of Gastroenterology: London, England, Balliere, pp. 487-510 (1984)

16. J.B. Thompson et al., JLCM 73:521-530 (1969)

17. O. Hernell et al.: Human milk enzymes with emphasis on the lipases, in Lebenthal E (ed): Textbook of Gastroenterology and Nutrition in Infancy. New York, NY. Raven, pp 209-217 (1989).

18. M.C. Carey et al., Annu. Rev. Physiol. 45:651-677 (1983).

19. P.J. Thomas, Gastroenterology 62:430-435 (1972).

20. H.V. Ammon and S.F. Phillips, Gastroenterology 65:744-749 (1973).

21. T.S. Gaginella et al., DDS 20:1171-1177 (1975).

22. J.H. Weisburger and E.L. Wynder. Etiology of colorectal cancer with emphasis on mechanism of action and prevention. In: V.T. DeVita et al (eds.). Important advances in oncology, J.B. Lippincott, Philadelphia, pp. 194-220 (1987)

- H.L. Newmark et al., JNCI 72:1323-1335 (1984)
- M.J. Hill et al., Lancet, ii:185-186 (1987)
- B.D. Reddy et al., Cancer Res. 37:3238-3242 (1977)
- P. Senesse et al., Gastroenterology, 108:A536 (1995).

23. [Hl. Holubec et al., Gastroenterology 106:A393 (1994); P.K. Bamberger et al., Gastroenterology 108:A447 (1995); D. Earnest, Gastroenterology 108:A463 (1995); R. Wali et al., Gastroenterology 108:A550 (1995); D. Peters et al., Gastroenterology 110:A576 (1996); T. Ochsenkühn et al., Gastroenterology 110:A571 (1996)].

24. Experiments reported by Reddy et al. have shown that CA, CDCA, DCA and LiCA, but not cholesterol, cholesterol epoxide, triol or their microbial products exert a tumor promoting effect in MNNG-induced colon carcinogenesis.

25. According to the work of L.L. Shekels et al. [JLCM 127:57-66 (1996)] BAs do not stimulate cell growth in undifferentiated or differentiated colon cancer cells lines, in contrast to normal colonic epithelium *in vivo*. BA cytotoxicity correlated with the relative hydrophobicity (TUDCA alters the cytotoxicity of DCA *in vitro*) *in vitro*.

26. It has been proposed that SCFAs, especially n-butyric acid, may have antineoplastic properties by inhibiting cell proliferation and inducing cell differentiation [J.J. Dang et al., Gastroenterology 106:A380 (1994); G. D'Argenio et al., Gastroenterology 106:A380 (1994); S..J.D. O'Keefe et al., Gastroenterology 108:A520 (1995); M. Barshishat, Gastroenterology 110:A489 (1996); L.J. Guyver et al., Gastroenterology 110:A524 (1996); I. Nordgaard, Gastroenterology 110:A569 (1996); B. Schwartz et al., Gastroenterology 110:A591 (1996); W. Scheppach et al., Gastroenterology 110A589 (1996); F. Richter et al., Gastroenterology 110:A583 (1996)].

27. Analysis of samples for BrdU and PCNA was conducted at the MD Anderson Cancer Center, Houston, TX. Two subjects' (#4 and #9) biopsy specimens were not scorable for the BrdU marker at discharge.

Analysis of samples for WCMC was conducted at Denver Dept. of Veteran Affairs Med. Center, Univ. of Colorado Sch. of Med., Denver, CO.

28. Fecal samples were analyzed by Medi-Lab, Copenhagen, Denmark.

29. In dairy cows, based on disappearance of LCFA from the large intestine, P.D. Møller [Acta Vet. Scand., Suppl 86:222-224 (1989)] has presented data that indicate either a transcellular absorption of LCFA from the large intestine or an oxidation and shortening of FA by bacteria in the hindgut for their energy supply. Studies on human colonic absorption of LCFA are not available.

30. Listed below are pertinent although selective references to the relationship between BAs and colon cancer:

- C.C. Boring et al., CA Cancer J. Clin. 44:7-26 (1994)
- D.J. Ahnen, J. Cell Biochem. 16G(Suppl):143-150 (1992)
- E. Bayerdörffer et al., Gastroenterology 104:145-151 (1993)
- E. Bayerdörffer et al., Digestion 55:121-129 (1994)
- J.W. Cook and G.A. Hazelwood, Chem. Ind. Rev. 11:758-759 (1933)
- M.J. Hill; Mechanisms of colorectal carcinogenesis IN: Diet and Human Carcinogenesis, J.V. Joossens (Ed.), Elsevier Scientific Publishers, pp 149-163 (1985)
- M.J. Hill, Eur. J. Cancer Prev. 1(Suppl 2):69-72 (1991)
- B.S. Reddy and E.L. Wynder, Cancer, 39:2533-2539 (1977)
- N. Tanida et al., Gut 25:824-832 (1984)
- M.J. Hill et al., Br. J. Surg. 72:5123-5124 (1985)
- B.S. Reddy et al., Cancer Res. 37:3238-3242 (1977)
- M. Wilpart et al., Carcinogenesis 4:45-48 (1983)
- K. Suzuki and W.R. Bruce, JNCI 76:1129-1132 (1986)
- J. Summerton et al., Digestion 31:77-81 (1985)
- B.S. Reddy et al., Prev. Med. 17:432-439 (1988)
- I.P. van Munster et al., Eur. J. Cancer Prev. 1(Suppl 2):35-44 (1991)
- B.S. Reddy et al., Gastroenterology 102:1475-1482 (1992)
- I. Makino et al., J. Lipid Res. 19:723-728 (1978)

31. This is also true for neoplasia in UC. Woolrich et al. studied the subsite distribution of dysplasia among patients with long-standing UC. Of 28 sites in which colonoscopy surveillance biopsy showed the presence of dysplasia, 19 (68%) were in the rectosigmoid colon. Although colon cancer mainly occurs in the distal part of the colon, cytolytic activity of fecal water is higher and AP activity (indicating degree of epithelial damage), is more pronounced after right than after left hemicolectomy. [This may explain in part the preponderance of tumors in the distal colon as compared to the proximal colon, especially after hemicolectomy [J.H. Kleibeuker et al., Gastroenterology 106:A403 (1994).] Right sided location as well as earlier age of adenoma diagnosis in probands are independent risk factors for an increased familial risk of colorectal cancer [A.G. Zauber et al., Gastroenterology 106:A455 (1994)]. In a study of colonic mucosal proliferation and DCA, T. Ochsenkühn et al. [Gastroenterology 110:A571 (1996)] found that serum DCA levels correlated significantly with the respective proliferation rates in the individual colonic segments (ascended, descended and sigmoid) but no correlation was found for the rectum and the cecum. Finally a historical prospective study conducted among residents of Rochester, MINN who underwent cholecystectomy concluded that the statistically significant increase in relative risk was observed only in women and was more marked for right-sided colon cancer [D.A. Linos et al., Lancet ii:379-381 (1981)]. Ornithine Decarboxylase activity (proposed as a marker of uncontrolled cell proliferation), was significantly higher than in the left colon or rectum [S. Civitelli et al., Gastroenterology 110:A504 (1996)].

32.

- B.N. Ames and L.S. Gold, Science 249:970-971 (1990)
- Ibid. Proc. Natl. Acad. Sci, U.S.A. 87:7777-7781 (1990)
- S.M. Cohen and L.B. Ellwein, Science 249:1007-1011 (1990)
- S.M. Cohen et al., Mod. Pathol. 4:371-382 (1991)
- S.M. Cohen and L.B. Ellwein, Cancer Res. 51:6493-6505 (1991)
- S. Preston-Martin et al., Cancer Res. 50:7415-7421 (1990)
- P. Grasso and M. Sharratt, Annu. Rev. Pharmacol. Toxicol. 31:253-287 (1991)

33. Colonic preparation with oral senna extract (anthraquinone glycosides) should be avoided when proliferation studies of the colon are to be performed [J.H. Kleibeuker et al., JNCI 87:452-453 (1995)].

34. The COX-2 gene has been shown to be expressed at high levels in 85% of human adenocarcinomas and 45% of human adenomas. COX-2 expression is increased in intestinal tumors that develop in Min mice and carcinogen-treated rats. Treatment of these animals with many different NSAIDs results in a marked decrease in tumor multiplicity. Together, all these results make it likely that COX-2 may be involved in the adenoma-to-carcinoma sequence of events and that increased expression of COX-2 may result from an inability of the *APC* gene product to carry out its normal function. If this hypothesis proves to be correct, the clinical implications would be profound since new drugs have recently been developed that are highly selective COX-2 inhibitors that suppress polyp formation [M. Oshima et al., Cell 87:803-809 (1996); J. Vane, Nature 367:243-249 (1994)].

MEDICAL/CLINICAL REVIEW

OF

4-MONTH SAFETY UPDATE

NDA#: 20-766

SPONSOR: ROCHE

DRUG: ORLISTAT

DATE SUBMITTED: 4/1/98

DATE OF REVIEW: 4/10/98

REGULATORY RECOMMENDATION

NO ACTION INDICATED.

/S/ [redacted] 4/28/98

ERIC COLMAN, M.D.

CC: NDA ARCH
HESS/TROENDLE

The company has submitted a 2-page summary to satisfy the requirements for the 4-month safety update.

According to the sponsor there are no clinical studies being conducted in the United States. Ongoing studies include the Xendos Trial (Swedish Study) and approximately 32 Phase 3b studies.

The Xendos Study, which completed enrollment in December of 1997, is a double-blind, placebo-controlled, parallel-group multi-center study evaluating the ability of orlistat to prevent obese patients from developing NIDDM. This is a two-year study with approximately 3300 patients.

The Phase 3b studies are being conducted in Europe, Mexico, South America, Canada, and Australia. All studies are double-blind and placebo-controlled. Orlistat 120mg tid is the standard dosing regimen.

The sponsor claims that there is no unblinded safety data available for either the Xendos Study or for the Phase 3b studies. In particular, there have been no cases of breast cancer reported in any of these ongoing trials.

Medical Review
NDA 20-766
Four-Month Safety Update
Submitted 4/9/97
Reviewed 4/30/97

This safety update contains the following information:

1. Status of ongoing studies
2. Errata found in the Phase 3 studies
3. Final reports of 9-month rat colon studies

1. Status of Ongoing Studies

Five studies are ongoing, these studies were initiated after 11/27/96.
NP15480B - Interaction between orlistat with supplemental vitamin D
NP15481 - Interaction between orlistat with supplemental vitamin A
NP15491 - Effect of orlistat on mineral balance
M37001 - Effect of orlistat in obese patients with hypercholesterolemia
M37003 - Open-label extension of orlistat in obese patients with hypercholesterolemia

2. Errata found in phase 3 studies

There were minor, inconsequential changes made to the adverse event calculations and reclassifications of ECG abnormalities.

3. Rat colon studies (see pharmacology review)

GCR N-139,069 The results of this 9-month study in rats showed no adverse effects in the colon from orlistat-treated rats with respect to routine microscopy, cell proliferation, or incidence of aberrant crypt foci (ACF).

GCR N-139,068 The only finding of note was an increased incidence of ACF in female rats treated with 140 and 280 ppm of orlistat in their diet.

/S/ [redacted]

Eric Colman, M.D.

cc: NDA Arch
Hess/Colman/Troendle